*lox*P vs. *lox*K1 vs. *lox*K2

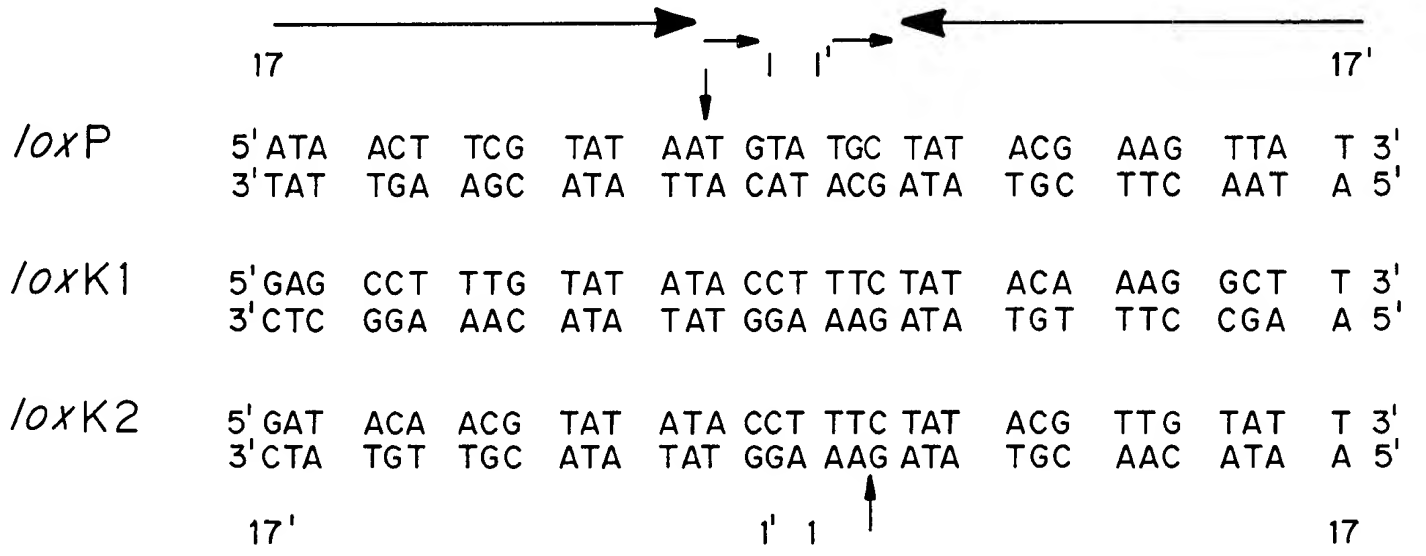


FIG. 1

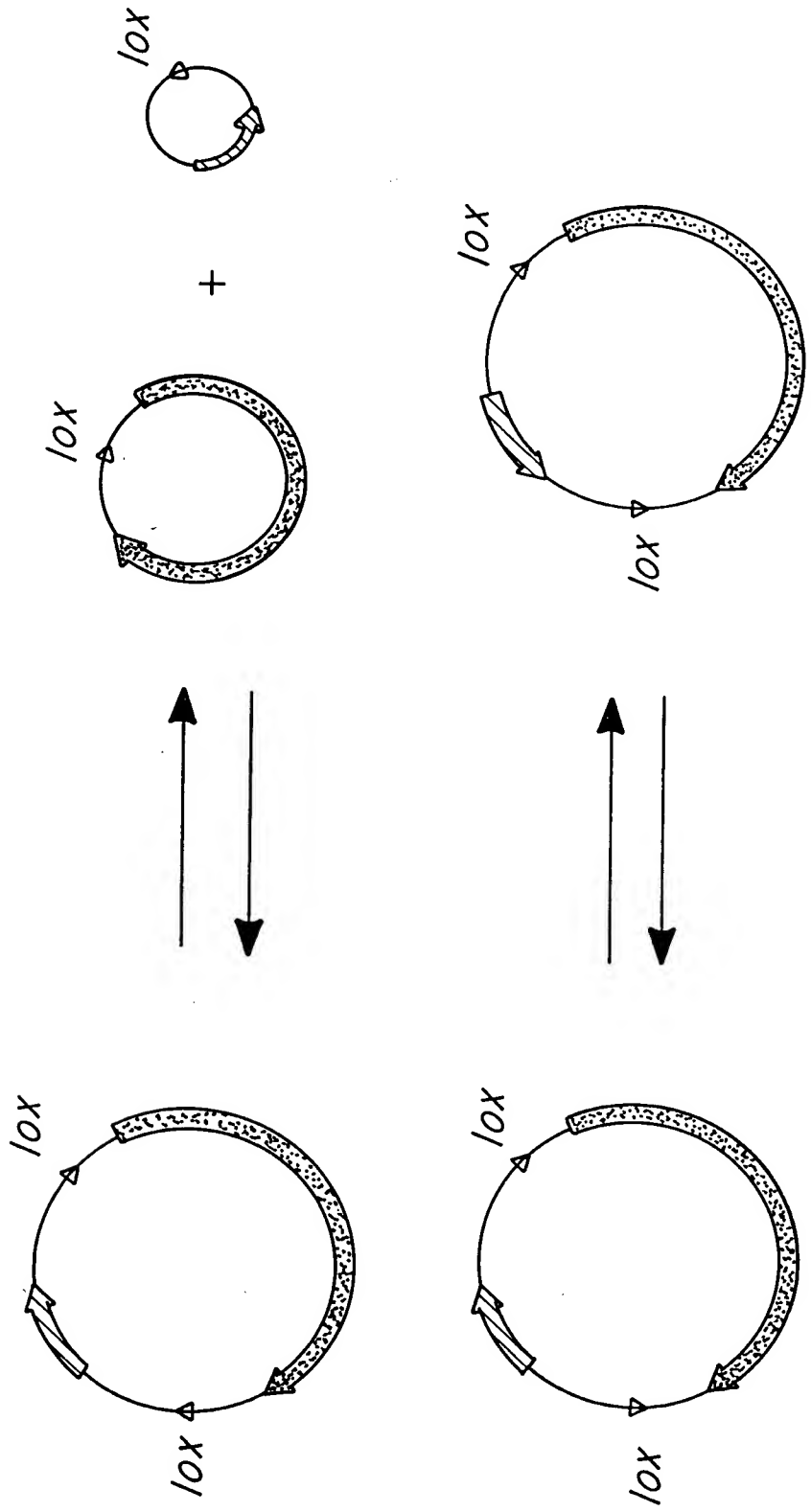
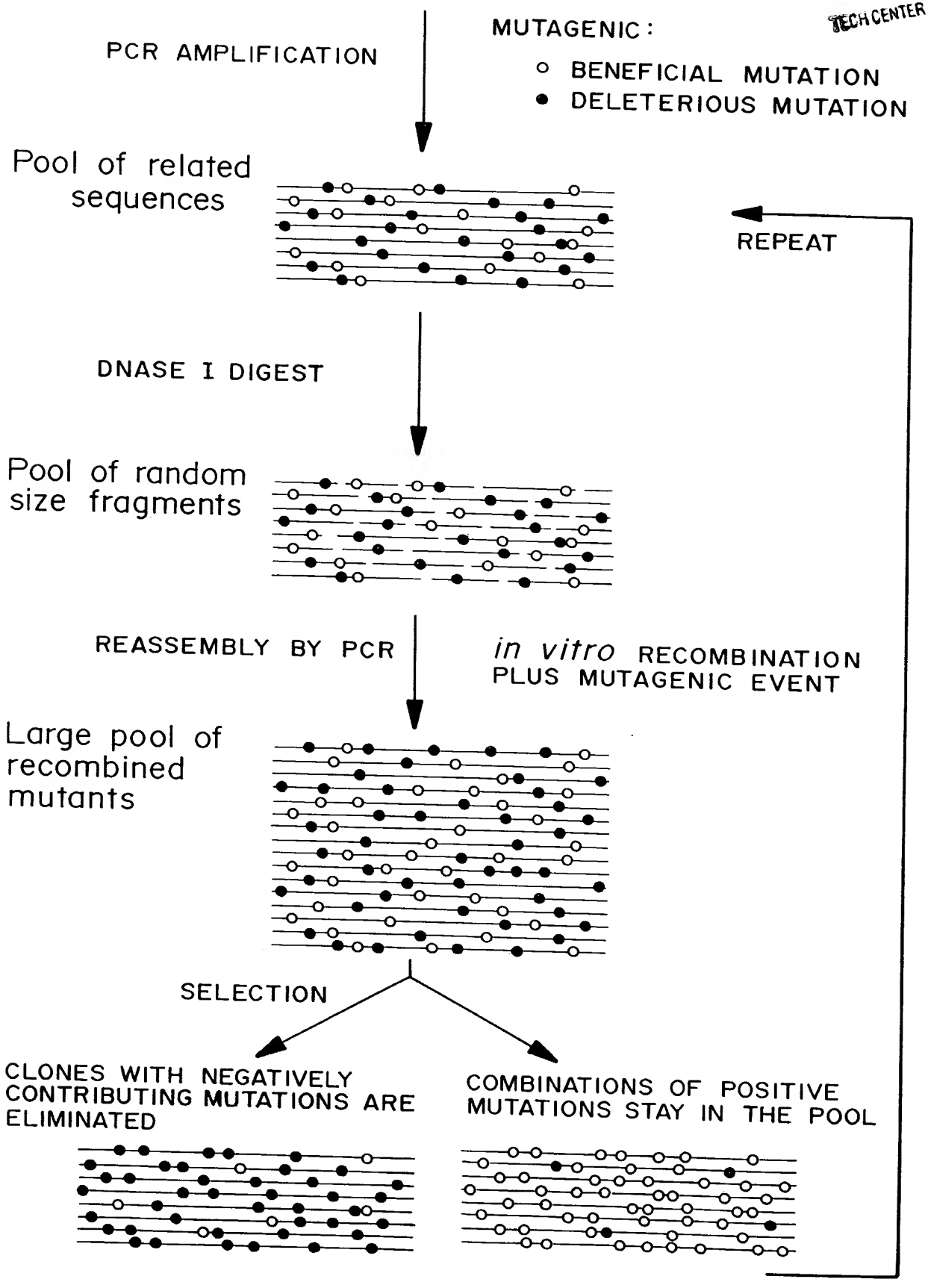


FIG. 2

FIG. 3

cre



U.S.N. 09/544,045
ORF 178

FIG. 4

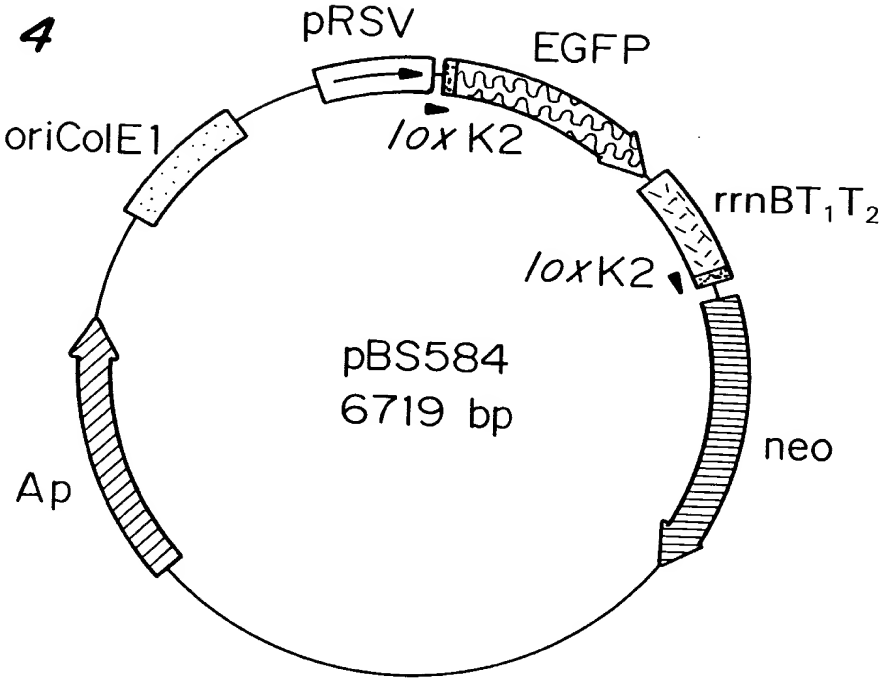


FIG. 6

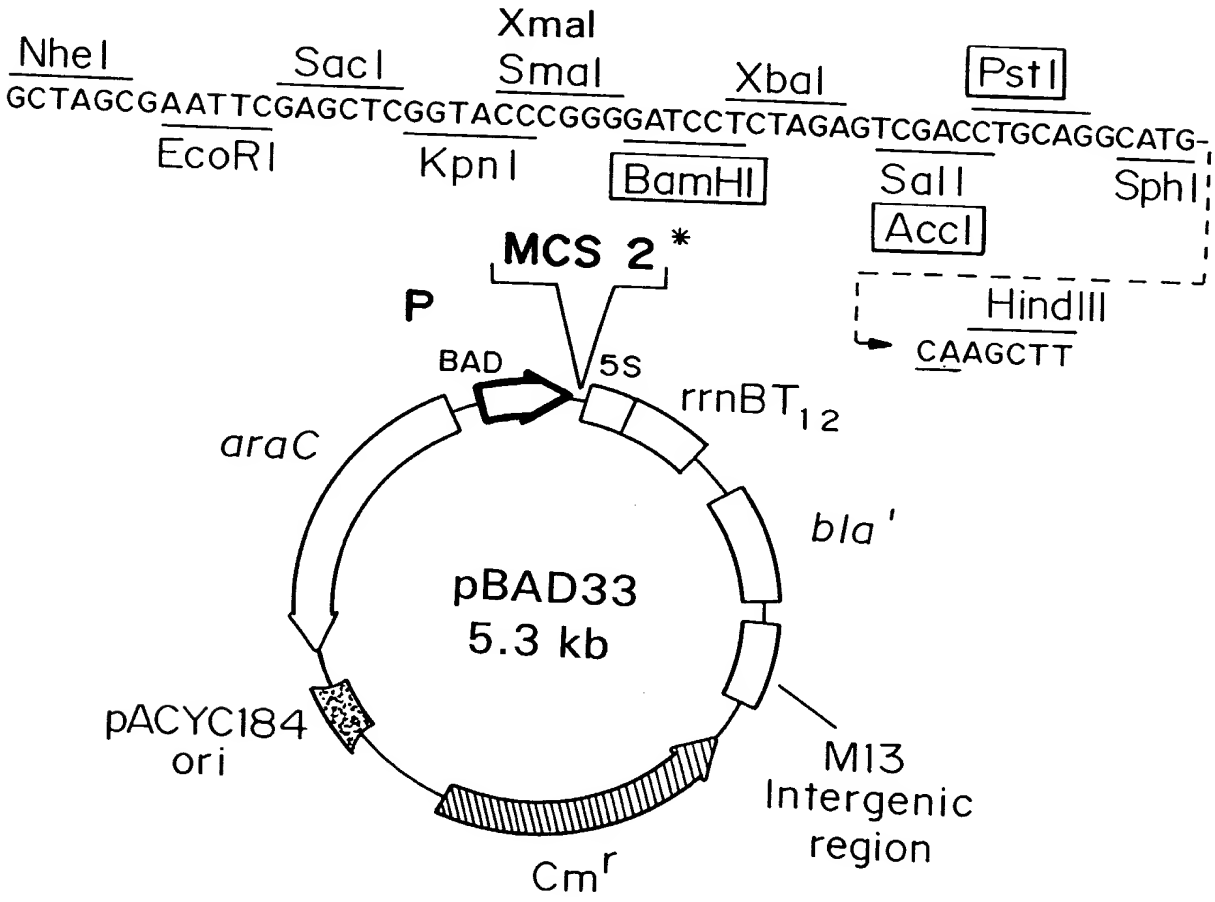


FIG. 5A

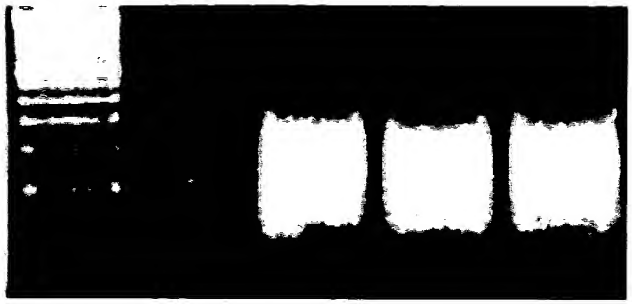
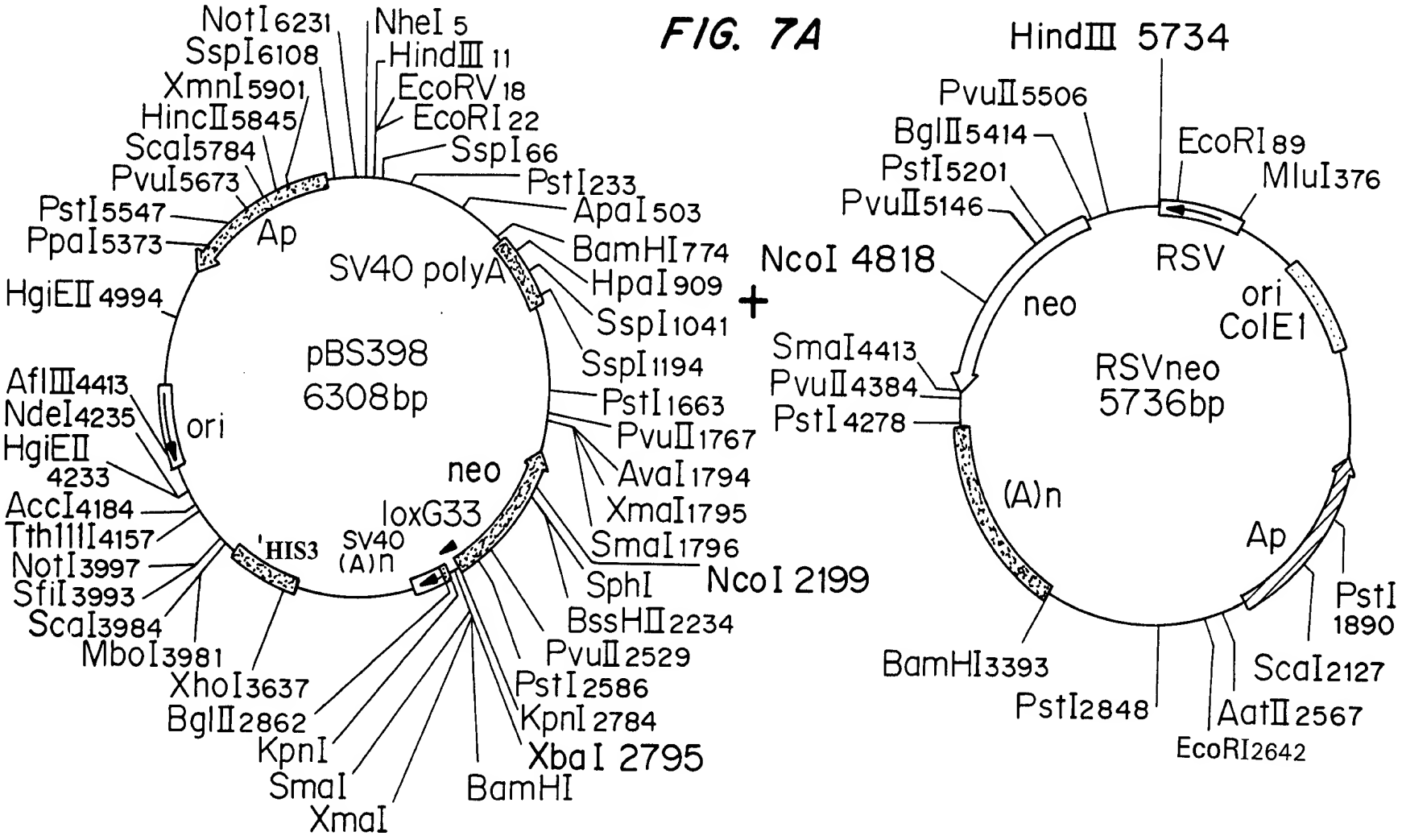


FIG. 5B

FIG. 5C

FIG. 7A



CONTINUED ON FIG. 7B

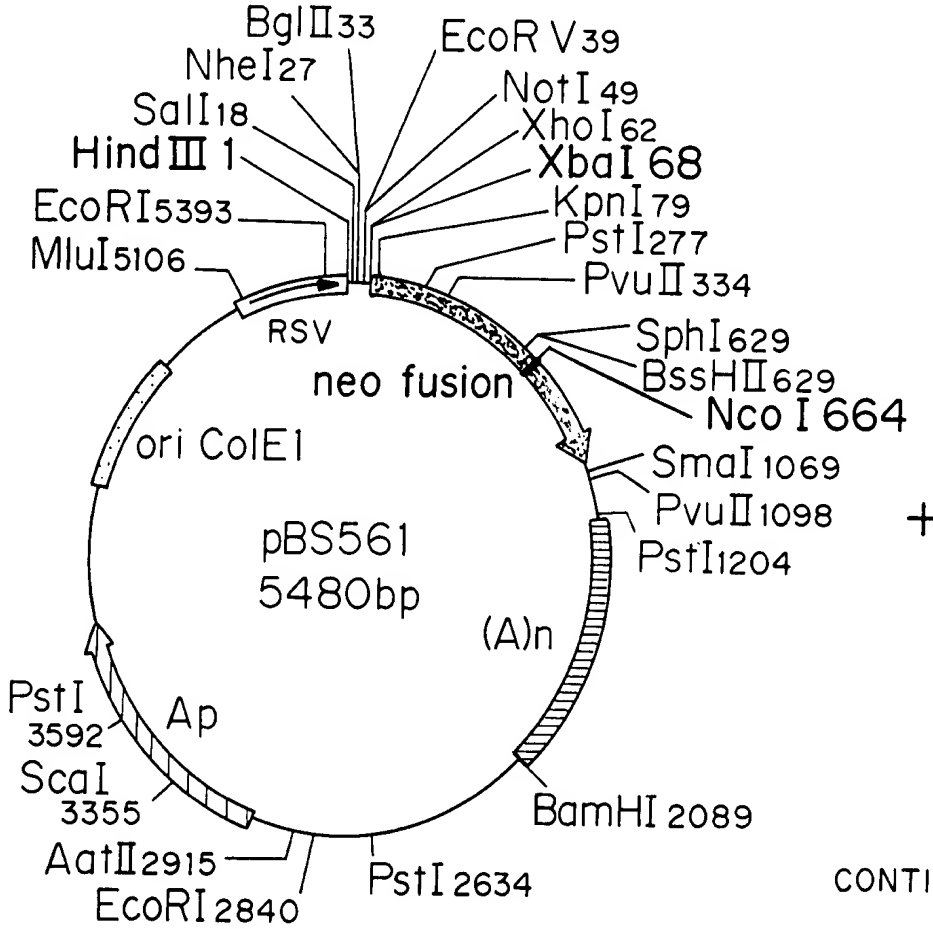
MCS for pBS 561-2 sets of oligonucleotides

HindIII SD Start *SalI* *NheI* *BglII*

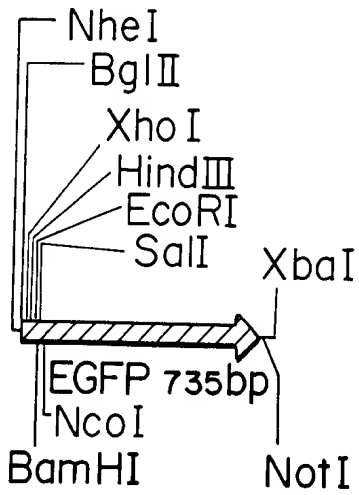
5' AGC TTG GAG GCT ATC ATG TCG ACC AAG CTA GCA 3'
3' AC CTC CGA TAG TAC AGC TGG TTC GAT CGT CTA G 5'

BglII *EcoRV* *NotI* Stop *XhoI* *XbaI*

5' GAT CTG ATA TCT GCG GCC GCT GAC GTG ACT CGA GT 3'
3' AC TAT AGA CGC CGG CGA CTG CAC TGA GCT CAG ATC 5'



+



lox-oligonucleotides

+ 2X

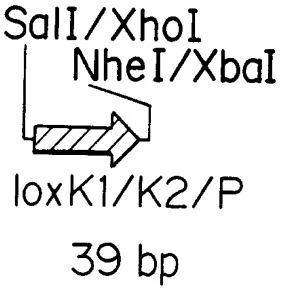


FIG. 7B

CONTINUED ON FIG. 7C

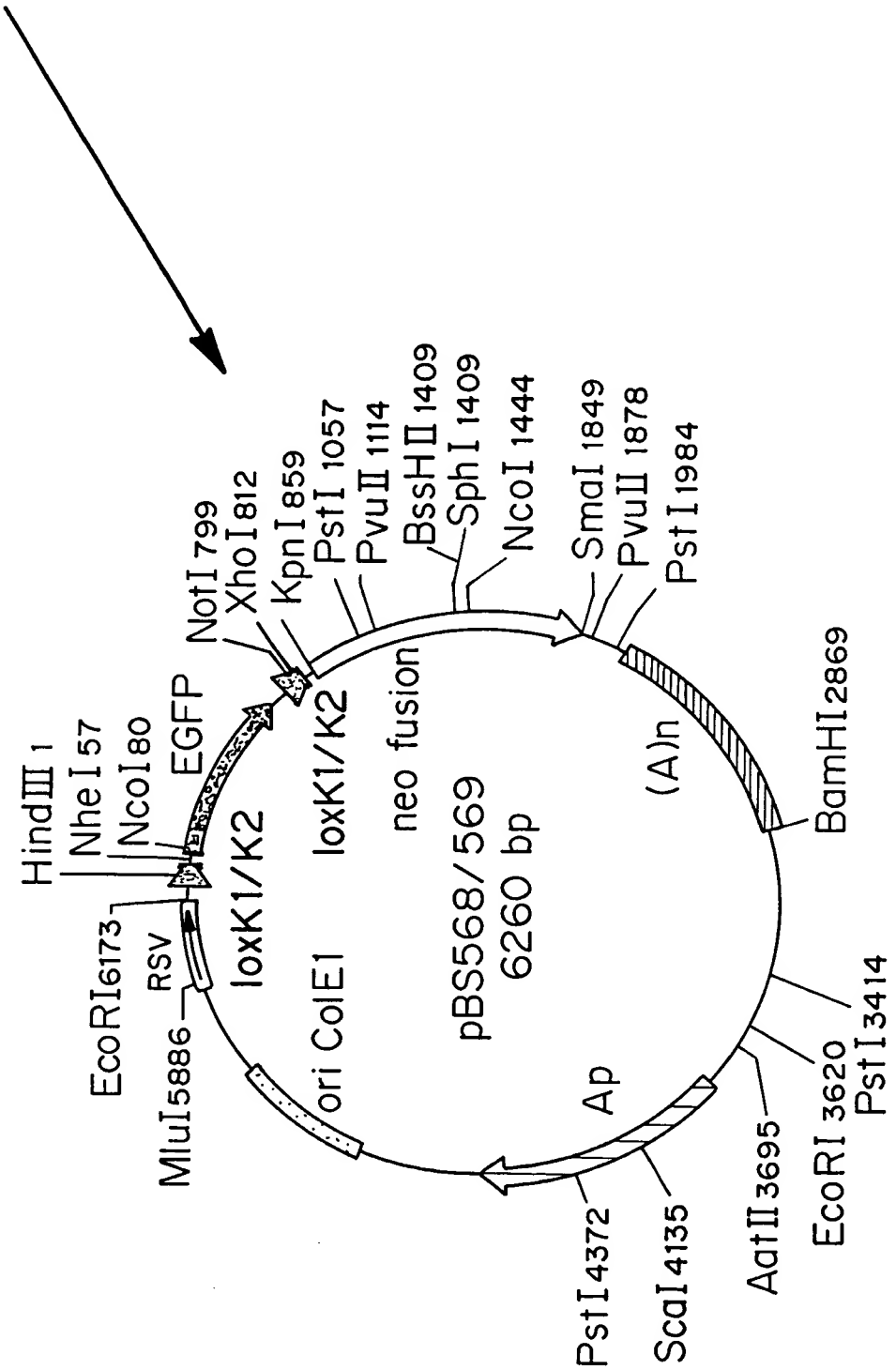
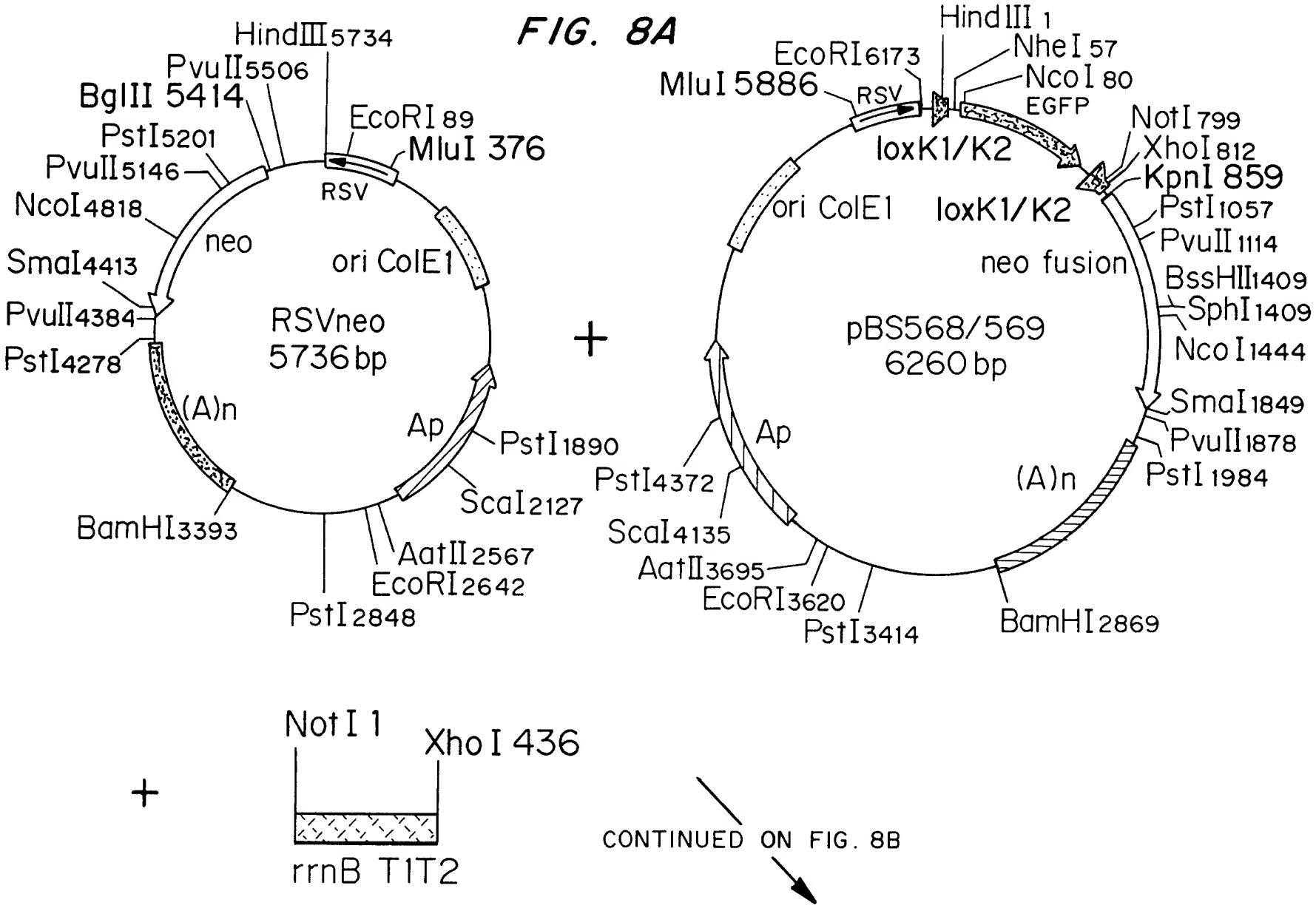


FIG. 7C

FIG. 8A



CONTINUED ON FIG. 8B

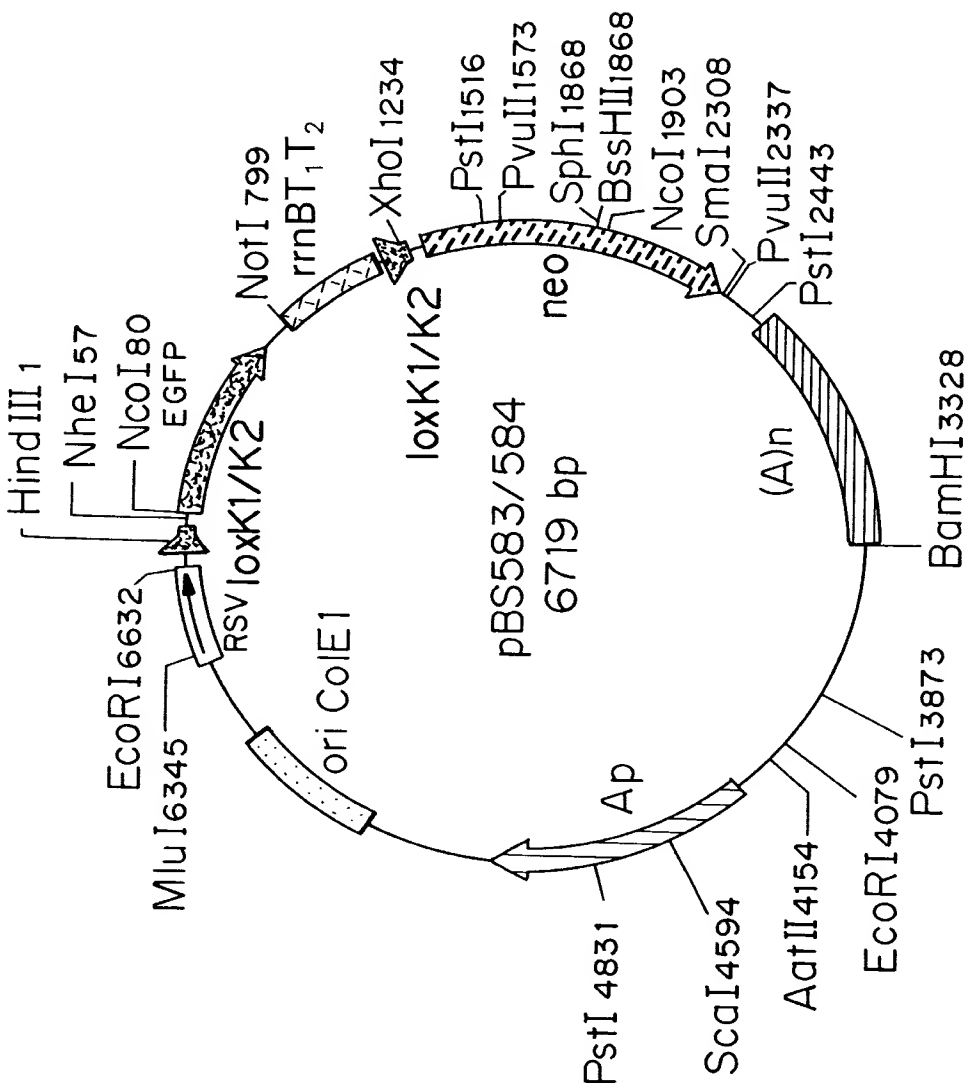


FIG. 8B

FIG. 9

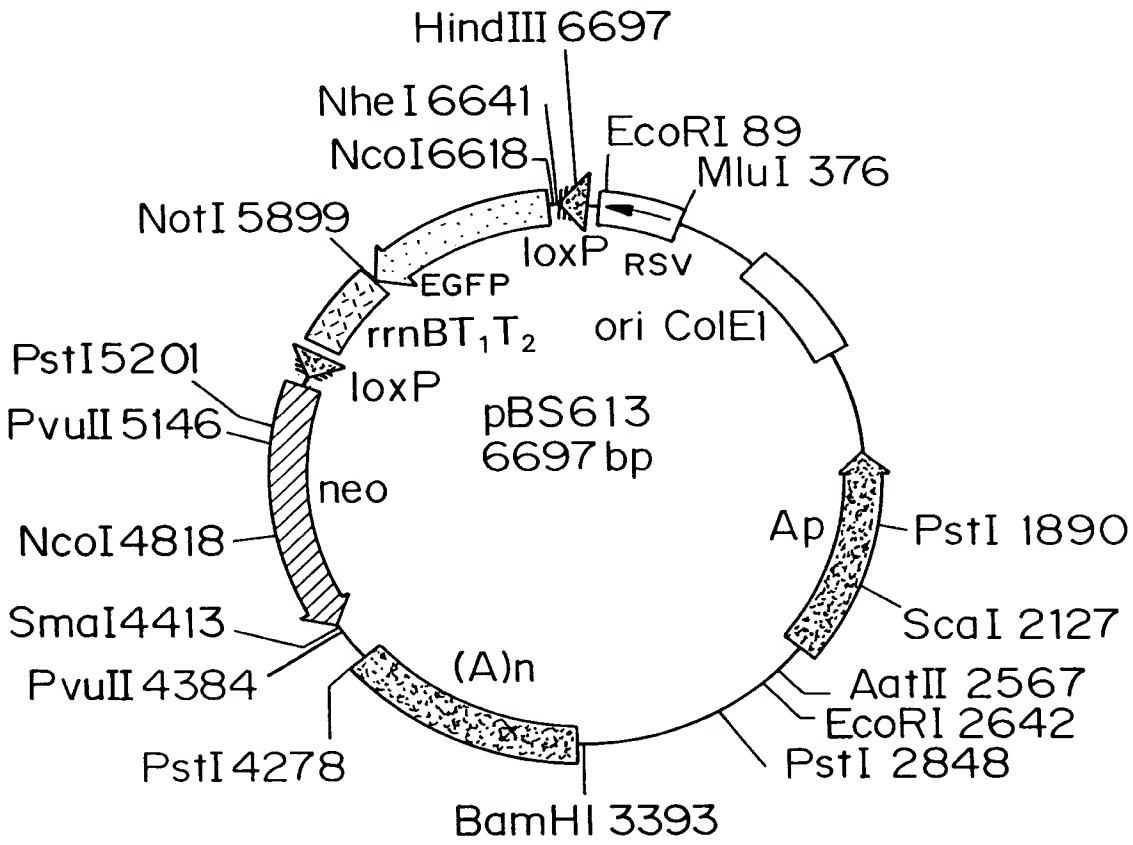
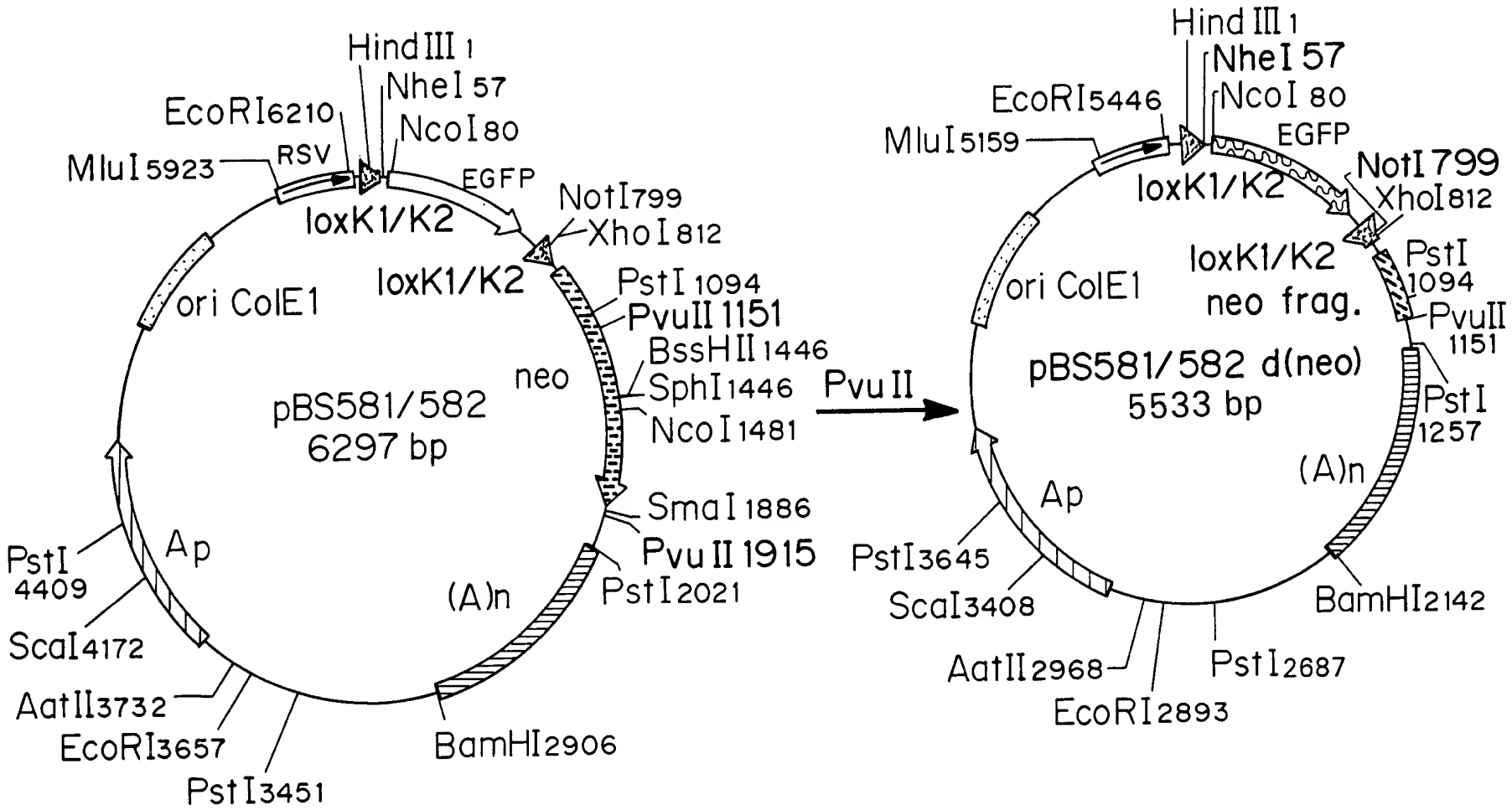
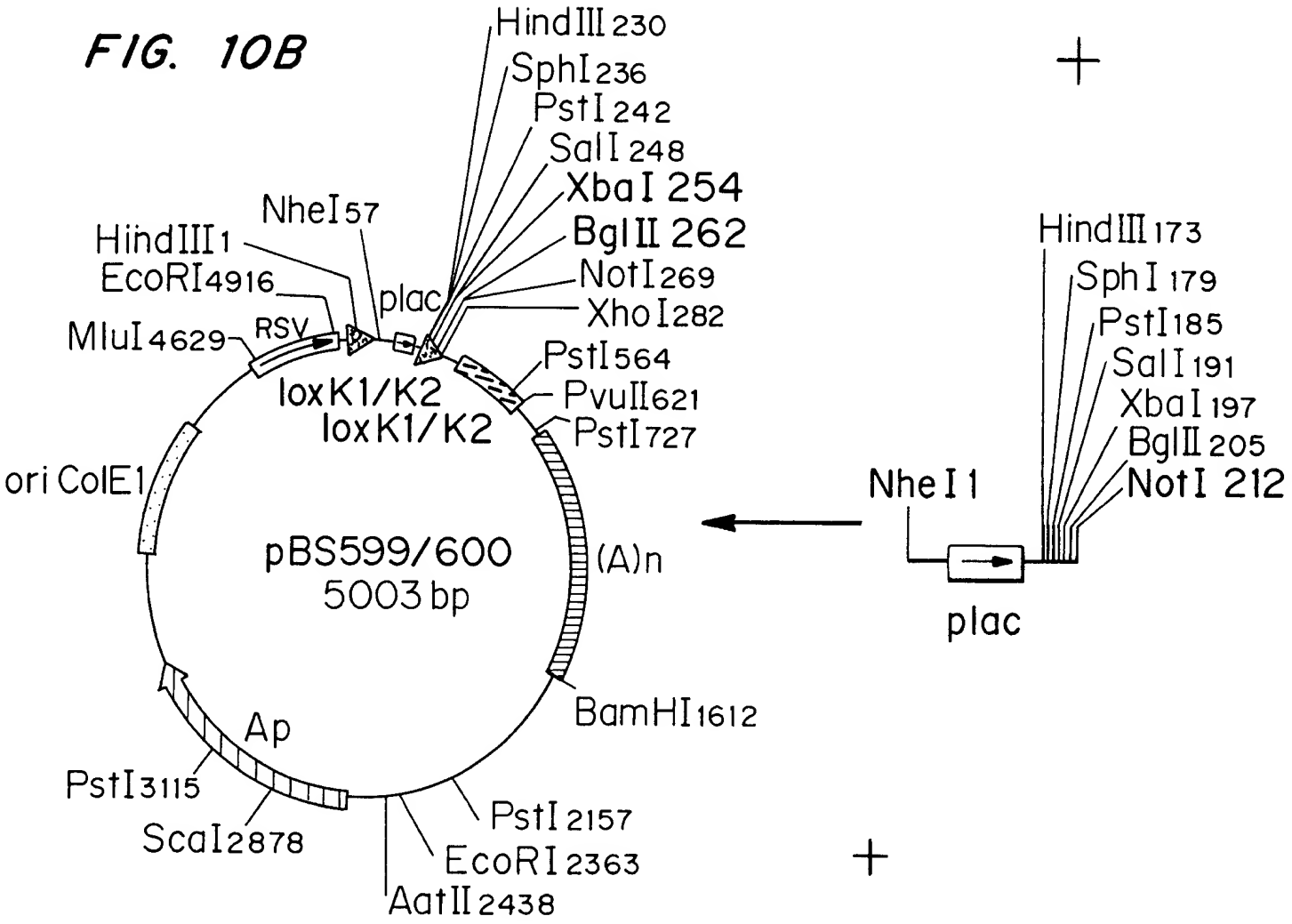


FIG. 10A



CONTINUED ON FIG. 10B

FIG. 10B



CONTINUED ON FIG. 10C

FIG. 10C

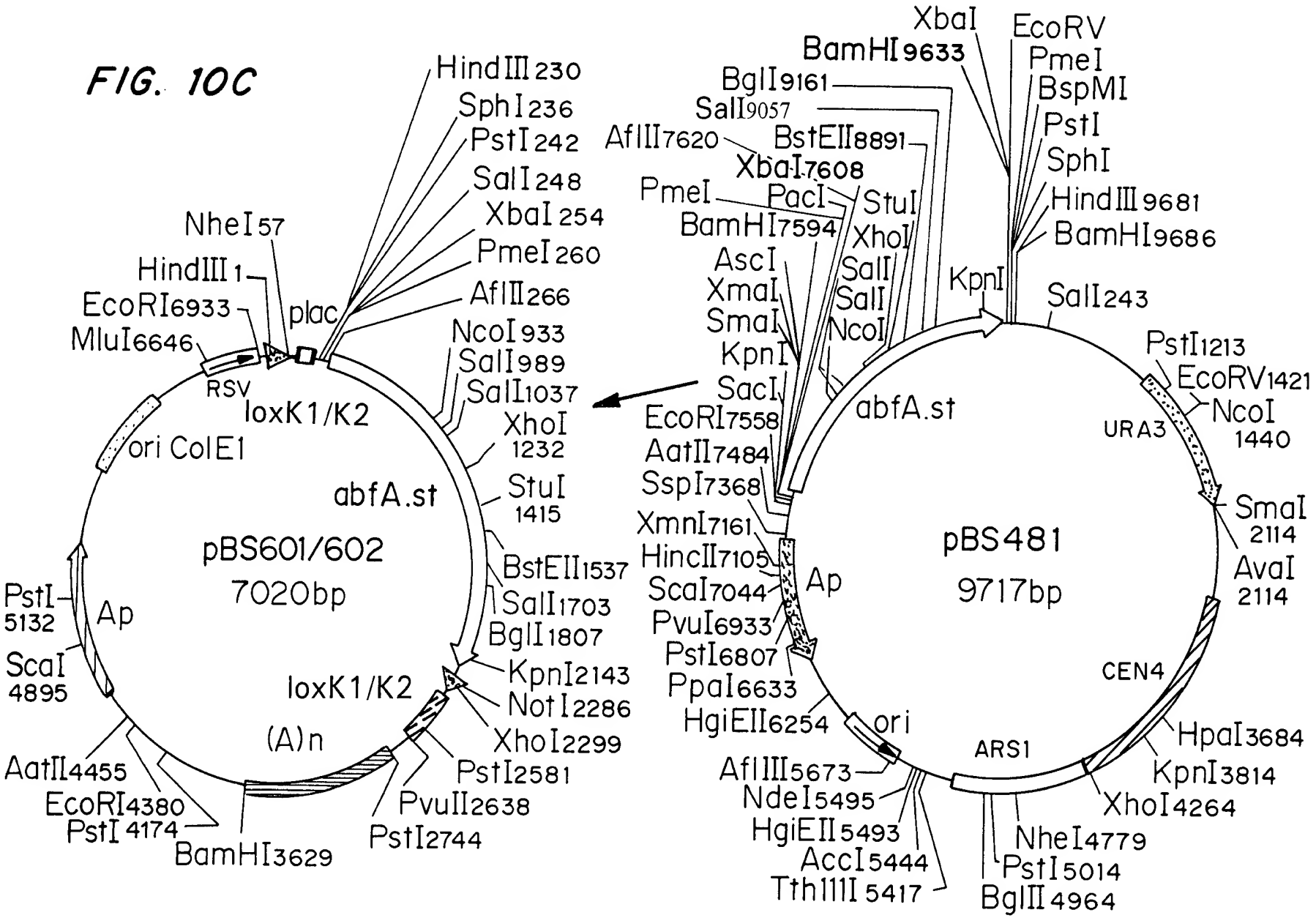

HindIII 230
SphI 236
PstI 242
SalI 248
XbaI 254
PmeI 260
AflII 266
NcoI 933
SalI 989
SalI 1037
XhoI 1232
StuI 1415
BstEII 1537
SalI 1703
BglI 1807
KpnI 2143
NotI 2286
XhoI 2299
PstI 2581
PvuII 2638
PstI 2744
BamHI 3629
PstI 4174
EcoRI 4380
AatII 4455
ScaI 4895
PstI 5132
Ap
ori ColE1
MluI 6646
EcoRI 6933
HindIII 1
NheI 57
RSV
plac
loxK1/K2
abfA.st
pBS601/602
7020bp
(A)n
loxK1/K2
XbaI
EcoRV
BamHI 9633
PmeI
BspMI
PstI
SphI
HindIII 9681
BamHI 9686
SalI 243
KpnI
BglI 9161
SalI 9057
AflII 7620
BstEII 8891
XbaI 7608
PmeI
PacI
StuI
BamHI 7594
XhoI
SalI
SalI
NcoI
AscI
XmaI
SmaI
KpnI
SacI
EcoRI 7558
AatII 7484
SspI 7368
XmnI 7161
HincII 7105
ScaI 7044
PvuI 6933
PstI 6807
PpaI 6633
HgiEII 6254
AflIII 5673
NdeI 5495
HgiEII 5493
AccI 5444
TthIIII 5417
BglII 4964
PstI 5014
NheI 4779
XhoI 4264
KpnI 3814
HpaI 3684
AvaI 2114
SmaI 2114
NcoI 1440
EcoRV 1421
PstI 1213
URA3
CEN4
ARS1
ori
Ap
abfA.st
pBS481
9717bp

FIG. 11

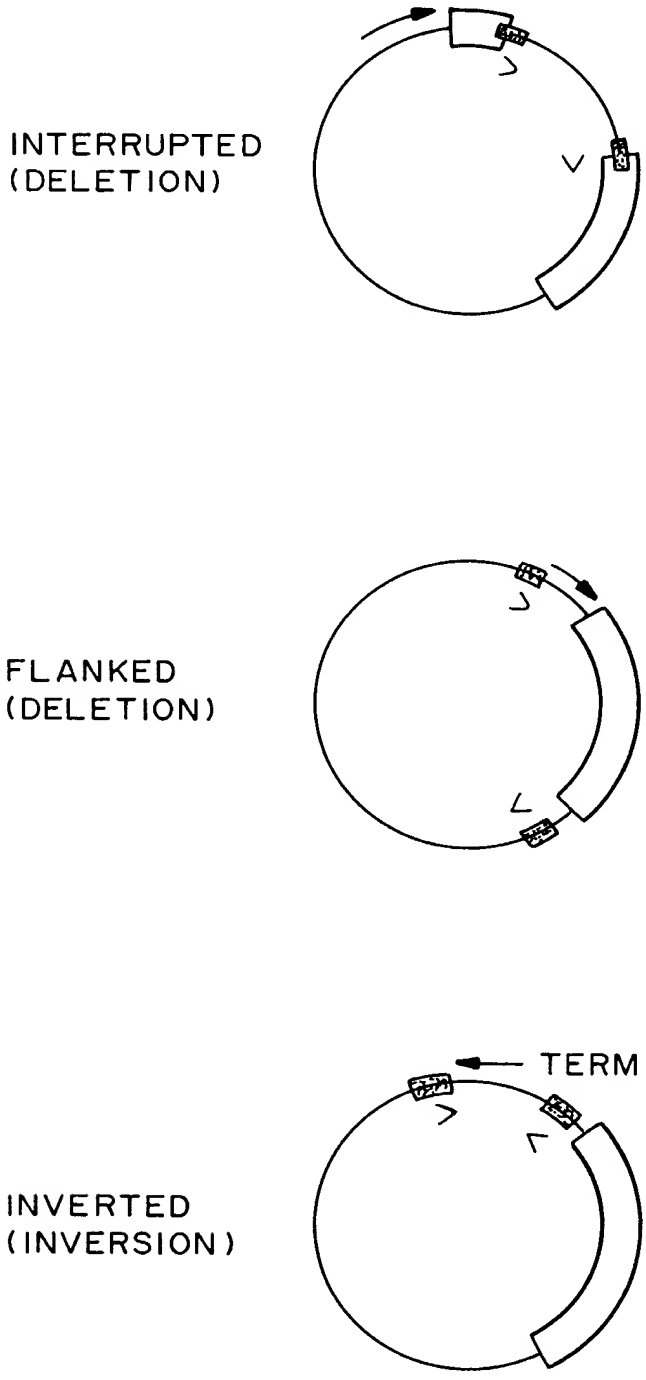
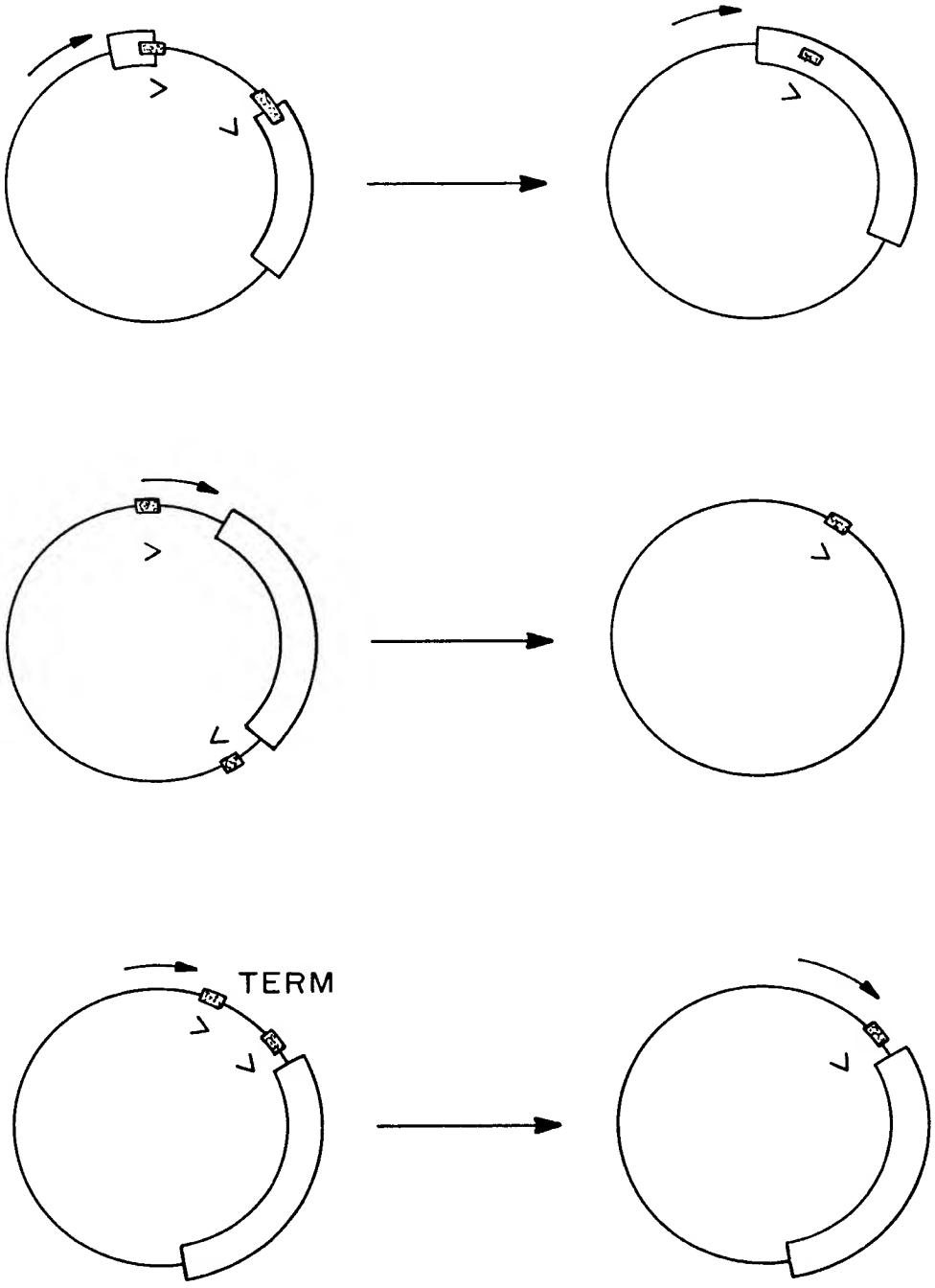
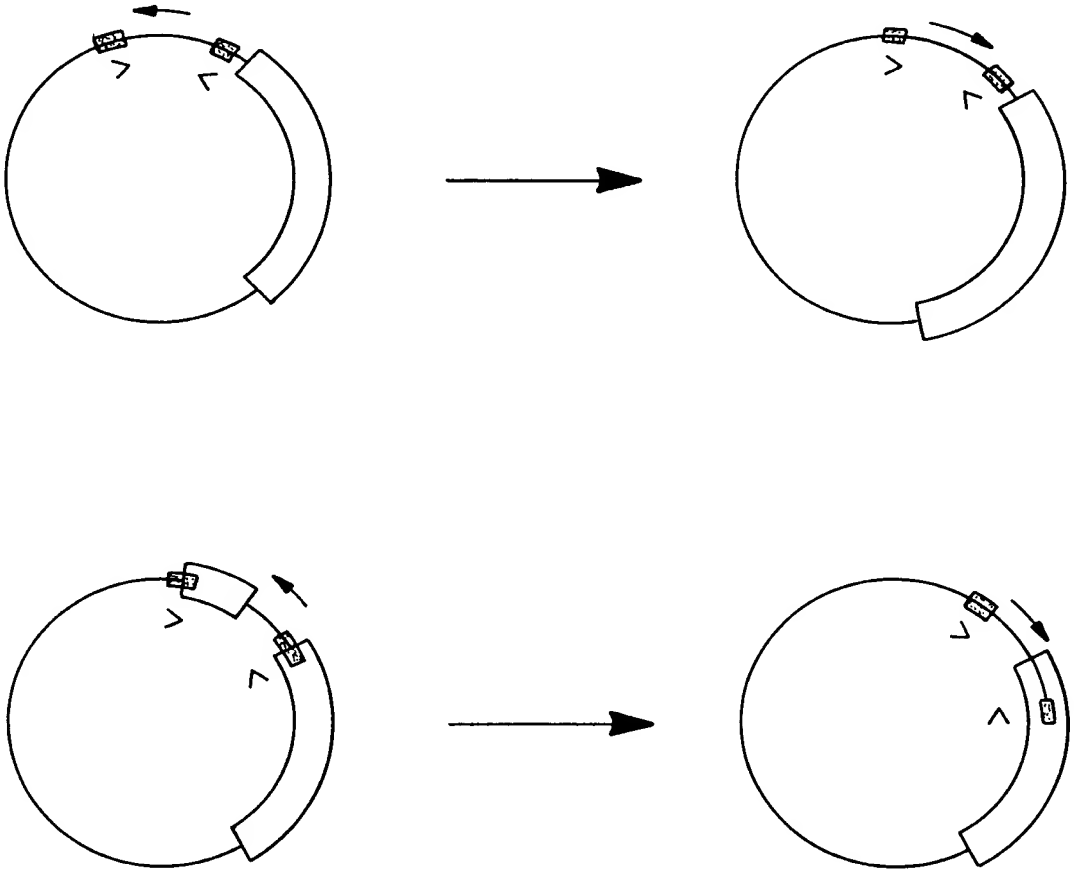


FIG. 12A



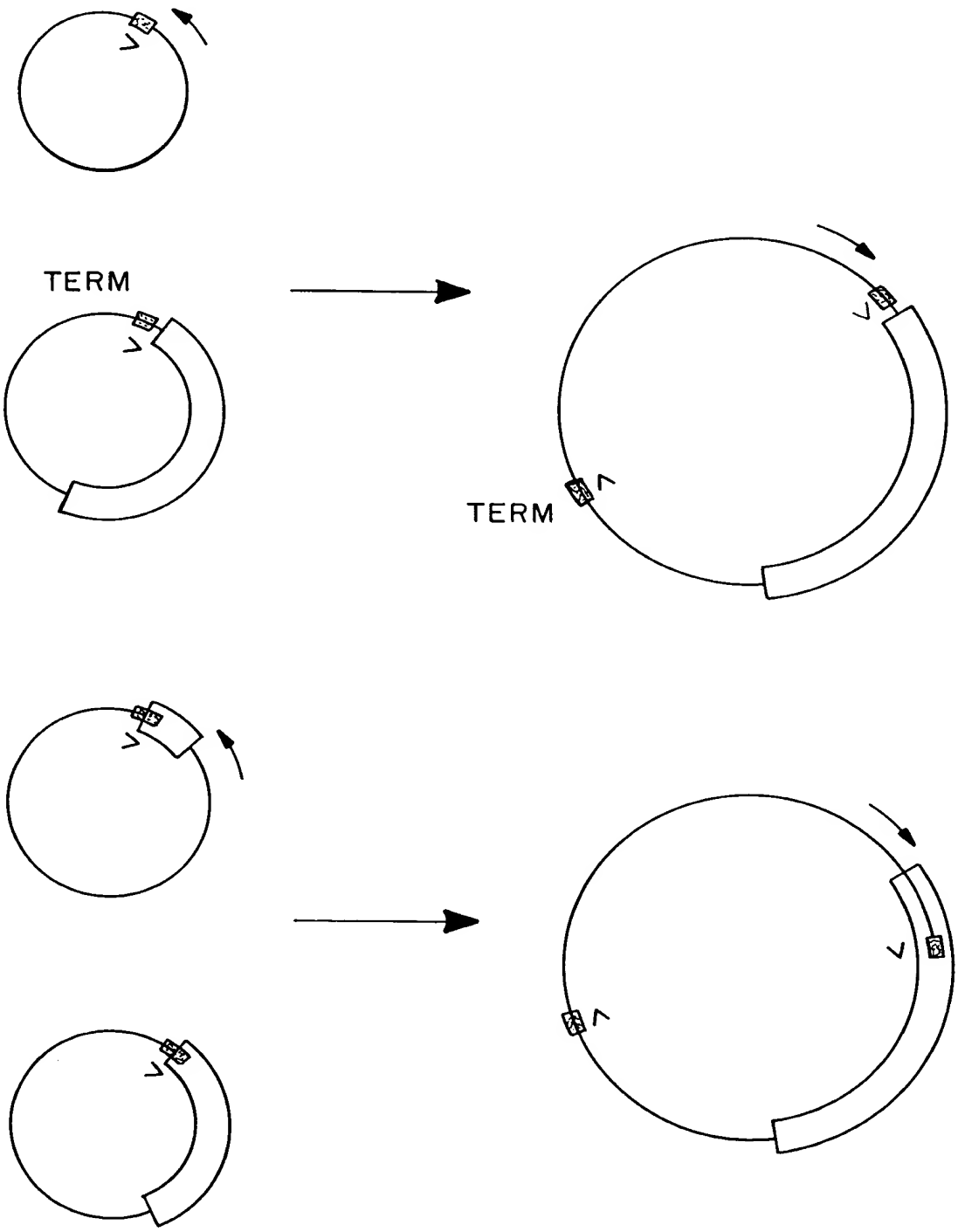
DELETION

FIG. 12B



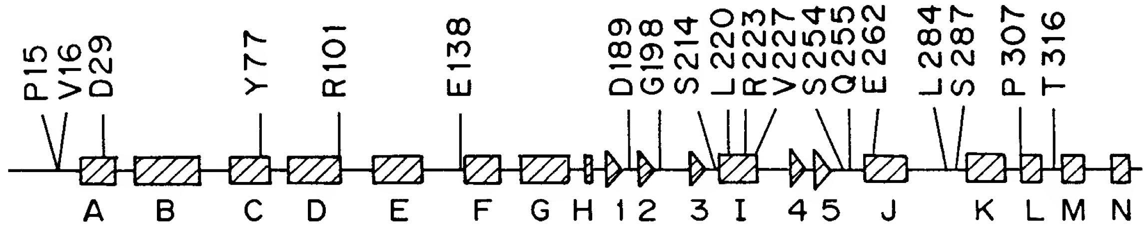
INVERSION

FIG. 12C



U.S.S.N. 09/544,045
OMRF 178

FIG. 13



R3M1 P - A - - - N - S - - I - - G - - - -

R3M2 P - - - - G - - - - - - G - G - S - S

R3M3 - I - - - - N S - - Q - - RG - S L -

R3M4 - - - C - K - - - - - - - - - Q - - -

R3M5 - - A - Q - - - - Q - - - - G - - - S

R3M6 - - - - - - - - - - - - - - G Q - - S

FIG. 14A

% of Cre-mediated recombination

	TT	GG	CC	AA
R3M3 Cre	97	82	67	90
E262G/D29A Cre	95	37	34	47
E262G/T316S Cre	98	21	14	32
E262G/D189N Cre	93	6	5	22
E262G Cre	90	20	10	28
wt Cre	95	0	0	0

loxP Halfsite ATAACTTCGTATA

FIG. 14B

% of Cre-mediated recombination

	loxP^2	*loxK2*^{2}	*loxP*-*loxK2*	*loxK1*^{2}	*loxP*-*loxK1*
R3M3 Cre	97	90	95	30	82
E262G/D29A Cre	95	47	80	2	30
E262G/T316S Cre	98	32	81	1	12
E262G/D189N Cre	93	22	71	1	17
E262G Cre	90	28	78	2	20
wt Cre	95	0	18	0	5

FIG. 15

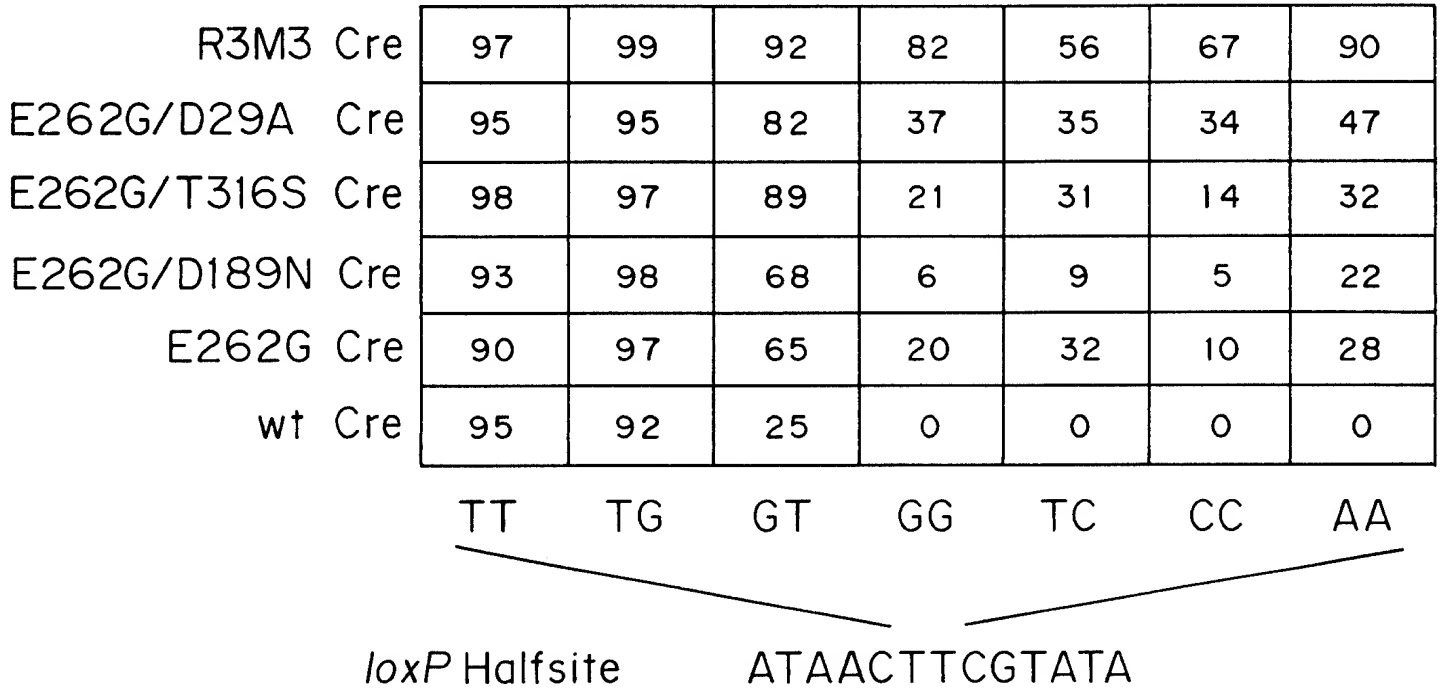
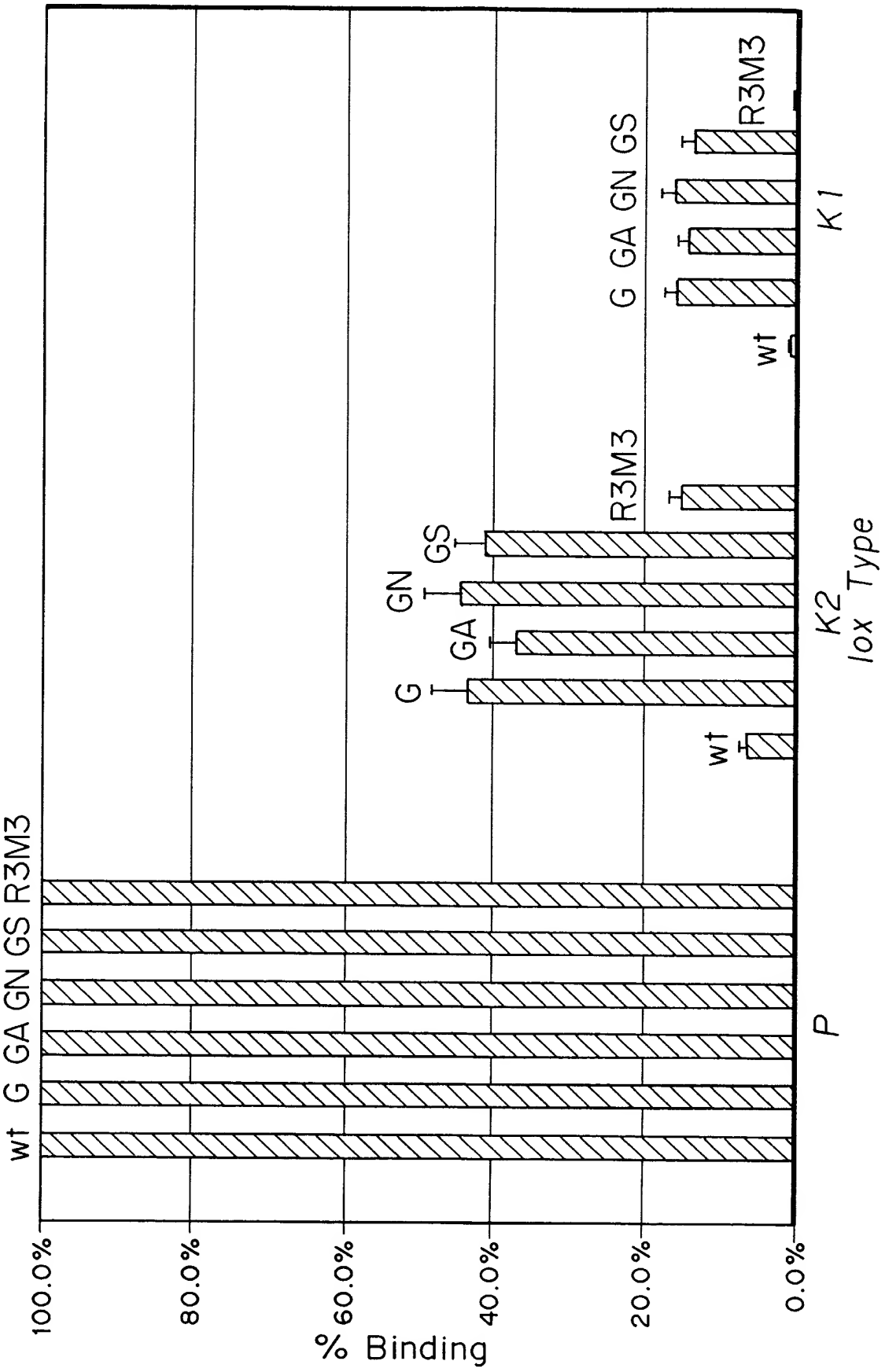

% Cre-mediated lox2 recombination
R3M3 Cre 97 99 92 82 56 67 90
E262G/D29A Cre 95 95 82 37 35 34 47
E262G/T316S Cre 98 97 89 21 31 14 32
E262G/D189N Cre 93 98 68 6 9 5 22
E262G Cre 90 97 65 20 32 10 28
wt Cre 95 92 25 0 0 0 0
TT TG GT GG TC CC AA
loxP Halfsite ATAACTTCGTATA

FIG. 16



wt FRT: GAAGTTCCTATTC TCTAGAAA GTATAGGAACTTC

Note - alternatively, a full site can also be used having 3 repeat elements:

GAAGTTCCTATTCCGAAGTTCCTATTC TCTAGAAA GTATAGGAACTTC

FRT-A1: GAAGTTCCTATTC TCTAGA tA GTATAGGAACTTC

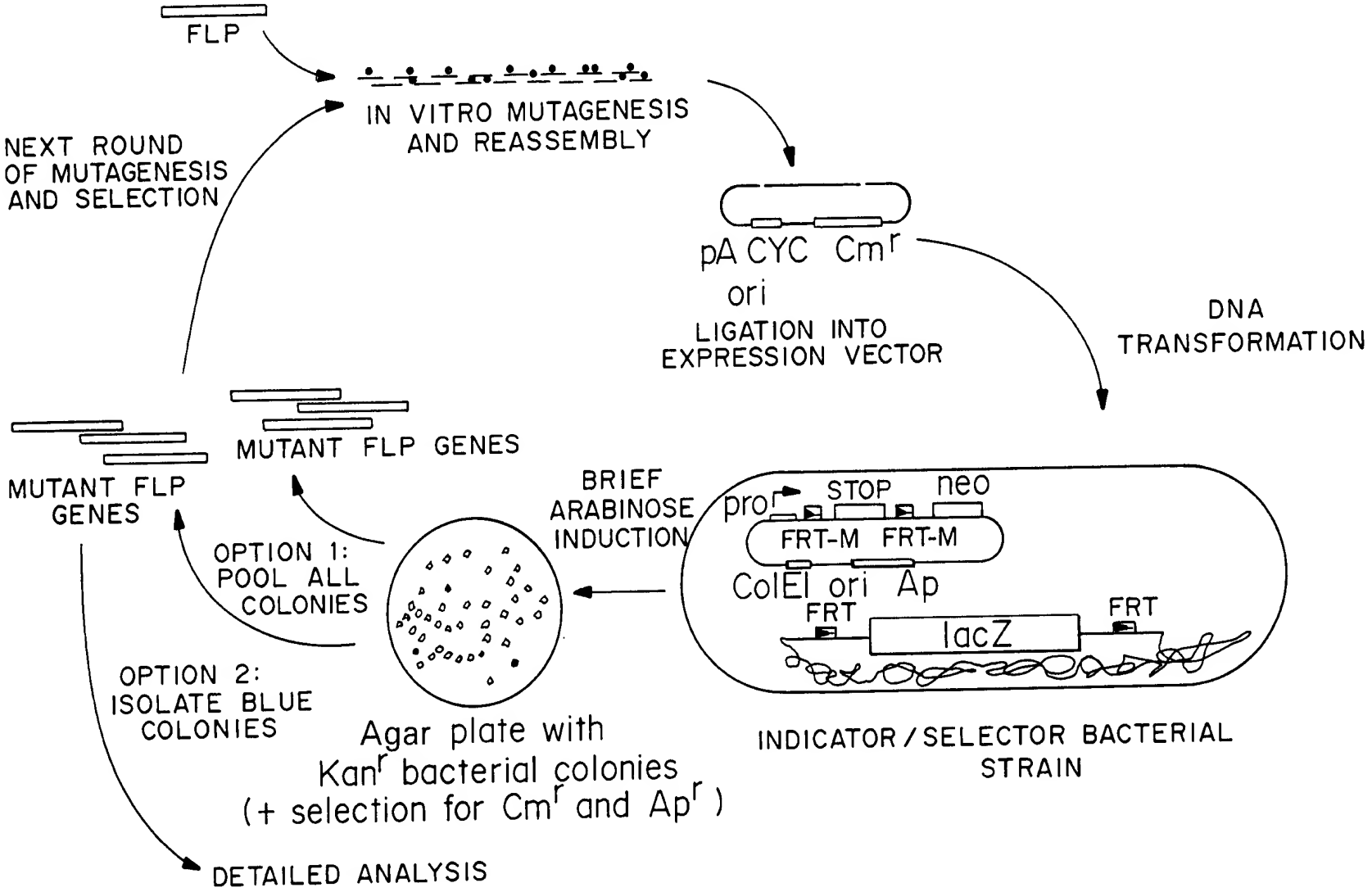
Spacer alteration

FRT-M: GAAGTTCaTATTC TCTAGA tA GTATAtGAACTTC

Symmetrical recognition

FIG. 17

FIG. 18


FLP
IN VITRO MUTAGENESIS AND REASSEMBLY
NEXT ROUND OF MUTAGENESIS AND SELECTION
pA CYC Cm^r
ori
LIGATION INTO EXPRESSION VECTOR
DNA TRANSFORMATION
MUTANT FLP GENES
MUTANT FLP GENES
BRIEF ARABINOSE INDUCTION
pro
STOP
neo
FRT-M FRT-M
ColEI ori Ap
FRT
FRT
lacZ
OPTION 1: POOL ALL COLONIES
OPTION 2: ISOLATE BLUE COLONIES
Agar plate with Kan^r bacterial colonies (+ selection for Cm^r and Ap^r)
INDICATOR / SELECTOR BACTERIAL STRAIN
DETAILED ANALYSIS

wt FRT: GAAGTTCCTATTC TCTAGAAA GTATAGGAACTTC

Note - alternatively, a full site can also be used having 3 repeat elements:

GAAGTTCCTATTCCGAAGTTCCTATTC TCTAGAAA GTATAGGAACTTC

FRT-A2: GAAGTTCCTATTC TCTAGA tA GTATAGGAACTTC

Spacer alteration

FRT-M2: GAAGTTaCTATTC TCTAGA tA GTATAGtAACTTC

Symmetrical recognition

FIG. 19